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Oxidized LDL stimulates the expression of TGF-β and fibronectin in human glomerular epithelial cells

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Oxidized LDL stimulates the expression of TGF-β and fibronectin in human glomerular epithelial cells. Abnormal lipid accumulation in glomeruli is a recognized early event in the development of glomerulosclerosis. The presence of LDL and scavenger receptors has recently been demonstrated in glomerular cells, including the visceral epithelial cells. To explore the possible molecular mechanisms of lipid-induced glomerular injury, the present investigation was conducted to examine the effects of oxidized LDL (ox-LDL) on the expression of transforming growth factor (TGF)-β and fibronectin by cultured human glomerular epithelial cells (GEC). Cultured GEC were exposed to human ox-LDL (0 to 100 μg/ml) for various time points. Ox-LDL induced a dose- and time-dependent increase in the expression of TGF-β mRNA. Actinomycin D, a transcriptional inhibitor, but not cycloheximide, a protein synthesis inhibitor, inhibited the response. GEC exposed to ox-LDL also demonstrated elevated levels of fibronectin mRNA. In addition, treatment of GEC with ox-LDL resulted in increased TGF-β and fibronectin protein expression as detected by immunocytochemistry. Addition of anti-TGF-β antibody significantly inhibited the increase in fibronectin message level induced by ox-LDL. These data suggest that ox-LDL stimulates matrix protein fibronectin in GEC by a mechanism involving expression of TGF-β. Thus, accumulation of lipids in human glomerular epithelial cells may contribute to the pathogenesis of glomerulosclerosis through TGF-β mediated mechanism(s).

Hyperlipidemia and lipoprotein abnormalities are associated with a variety of clinical and experimental renal diseases [1, 2]. In patients with the nephrotic syndrome, total serum cholesterol, triglycerides, phospholipids as well as apolipoprotein (apo) B, C, and E are elevated [3]. Qualitative alterations include an increased cholesterol-to-triglyceride ratio and increased cholesterol content both in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) [4, 5]. Abnormal lipid deposition is found in glomeruli with different lesions [6, 7]. In experimental models, hypercholesterolemia is also associated with glomerular lipid deposition and glomerulosclerosis [8–10]. Nephrotic animals fed a cholesterol-supplemented diet had much more profound morphologic injury, as evidenced by a higher percentage of glomerulosclerosis [11]. In addition, administration of lipid-lowering drugs to nephrotic animals ameliorated the adverse effects of hypercholesterolemia on glomerular structure and function [12–14]. Glomerular lipid deposits in rats contain mainly cholesterol and to a lesser extent triglycerides [8]. Analysis of renal tissue from experimental nephrotic animals revealed the accumulation of lipids in the mesangium and other glomerular cell types [15, 16]. Studies using immunohistochemical and immunoelectromicroscopical techniques revealed increased apo E immunoreactivity in glomerular visceral epithelial cells [10]. In tissues from several types of human glomerular diseases, apo B and E were distributed in droplets within glomerular epithelial cells [17, 18].

Recent investigations have shown that human glomerular mesangial and epithelial cells express receptors for LDL [17, 19–21]. Scavenger receptors [22], which can mediate the uptake of oxidized LDL, were detected in human glomerular cells by immunohistological studies [17]. This receptor-mediated binding and uptake of LDL was also found in rat mesangial cells [23, 24]. Furthermore, rat mesangial cells were reported to preferentially bind and take up oxidized LDL compared to LDL [25]. The accumulation of lipoproteins in glomerular cells may initiate a chronic inflammatory reaction and alter cell functions. Addition of LDL to mesangial cell cultures resulted in alterations in cell proliferation, prostaglandin synthesis, expression of growth factors and cytokines, and extracellular matrix protein production [25–30].

Transforming growth factor (TGF)-β, a multifunctional growth cytokine, has been implicated in the pathogenesis of glomerulosclerosis *in vivo* and in the modulation of the production of extracellular matrix proteins by glomerular mesangial cells and epithelial cells *in vitro* [31–34]. In glomeruli from hypercholesterolemic nephrotic animals increased fibronectin expression is associated with increased TGF-β expression [35]. LDL stimulates the expression of TGF-β mRNA in cultured mesangial cells [36]. The increased TGF-β bioactivity by mesangial cells exposed to LDL has been associated with enhanced fibronectin synthesis [28].

Glomerular epithelial cells are one of the major cell types of the glomerulus. They are a direct target of high concentrations of lipoproteins during nephrosis [21]. Both LDL and oxidized LDL receptors are documented on their surface membranes [17]. Human GEC exhibit receptor-mediated uptake of LDL and intermediate density lipoproteins [21]. However, the effects of lipoproteins on their functions, especially in regard to growth

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factor production and a fibrogenic mechanism have not been fully explored. To date there are no data published on the expression of TGF-β by human GEC. The present study was designed to examine the effects of lipoproteins on the expression of TGF-β and fibronectin in cultured human GEC, and to evaluate the effect of lipoprotein-induced TGF-β on expression of this matrix protein message.

Methods

Human glomerular epithelial cell culture

Human kidney tissue was obtained from macroscopically normal areas of nephrectomy specimens from renal tumor patients. Glomeruli were isolated by differential sieving of minced cortices, collagenase digested and plated, as previously described [37]. Early cellular outgrowths at 8 to 14 days were removed by trypsinization, filtered, and then replated. Glomerular epithelial cells were identified by their characteristic polygonal appearance as seen on phase-contrast microscopy, sensitivity to puromycin aminonucleoside, and staining for heparan sulfate proteoglycan and vimentin, but not for myosin, actin or factor VIII on immunofluorescence [38, 39].

Although it is not possible to determine specifically whether GEC in culture originate from visceral or parietal epithelium by current criteria [40, 41], we believe that the cells originating from decapsulated glomeruli after sieving, are most likely of visceral origin [39].

Human GEC (from 2 donors) were used between passages 5 and 9 and were maintained in Dulbecco's minimal essential medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; GIBCO, Grand Island, New York, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 15 mM Hepes, 5 μg/ml insulin, 5 μg/ml transferrin and 5 μg/ml selenium (ITS; Collaborative Biomedical Products, Bedford, MA, USA) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cells were grown in 100 mm petri dishes (Becton Dickinson, Franklin Lakes, NJ, USA) until subconfluence was reached.

Experimental treatments of GEC

Prior to all experiments GEC were placed in 5% Nu-Serum (a defined serum substitute containing 25% FBS, Collaborative Research) for 48 hours to provide a relatively lipoprotein deficient environment and to maximize lipoprotein receptor activity [42]. Then, cultures were exposed to various concentrations of native LDL and ox-LDL for the indicated times. LDL and ox-LDL were obtained from Biomedical Technologies Inc. (Stoughton, MA, USA). As supplied, LDL was isolated from human plasma and purified via ultracentrifugation to homogeneity as determined by agarose gel electrophoresis. The preparations contained negligible thiobarbituric acid-reactive moieties (TBARS) as determined by using a calorimetric assay for malondialdehyde (MDA). Ox-LDL was prepared using the oxidant Cu_2SO_4. The TBARS value of ox-LDL was 19.3 nM of MDA/mg protein. In separate experiments, GEC were incubated with ox-LDL in the presence of rabbit anti-human TGF-β antibody or nonspecific rabbit IgG (R&D Systems, Minneapolis, MN, USA) for 6 and 24 hours. The cultures were also treated with either actinomycin D (5 μg/ml), cycloheximide (10 μg/ml; both from Sigma), ox-LDL (50 μg/ml) and actinomycin (5 μg/ml), or ox-LDL (50 μg/ml) and cycloheximide (10 μg/ml).

Cell viability was determined by microscopic evaluation and by exclusion of trypan blue (Sigma). All treated-cells appeared to be healthy and there was no observable adverse effects on cellular morphology and trypan blue exclusion (> 95%).

RNA preparation and analysis

After treatment of the cells for the indicated times, total cellular RNA was extracted by the acid guanidinium thiocyanate phenol chloroform method [43]. RNA concentrations were determined using spectrophotometric absorbance at 260 nm. Fifteen μg RNA samples in ethidium bromide (1 μg/ml) were electrophoresed on a 1.2% agarose gel containing 0.66 M formaldehyde and subsequently blotted onto NYTRAN nylon membranes (Schleicher & Schuell, Keene, NH, USA) by capillary transfer. RNA was immobolized by baking 80°C for 30 minutes. The blots were examined under ultraviolet illumination to determine the position of the 28 s and 18 s ribosomal RNA bands and to assess the integrity of RNA. The blot was then sandwiched between two pieces of Schleicher & Schuell 589-WH qualitative filter paper and hybridized in a plastic bag in a solution containing 1 M NaCl, 0.05 M Tris-HCl, pH 7.4, 10% dextran sulfate, 1% sodium dodecyl sulfate, and 0.1 mg/ml salmon sperm DNA with addition of a human oligonucleotide probe for TGF-β1 (Oncogene Science, Inc., Uniondale, NY, USA), a human fibronectin cDNA probe (GIBCO BRL), and a human GAPDH cDNA probe (Clontech, Palo Alto, CA, USA) as a reference probe to allow for corrections in differences in RNA sample loading. The oligonucleotide probe was end-labeled with [^{32}P]-ATP and T_4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN, USA). The cDNA probes were labeled with [^{32}P]-dCTP, using a random-primed cDNA labeling kit (Boehringer Mannheim). After hybridization at 65°C for 20 hours, blots were washed twice in 2 × SSC-0.1% SDS at 25°C for 20 minutes and once in 2 × SSC-0.1% SDS at 65°C for 30 minutes. The blots were then exposed to Kodak X-O MAT film with enhancing screens at −70°C to develop autoradiograms. The quantitative densitometry was performed on autoradiographs with a computer-based measurement system. The mRNA levels for TGF-β1 and fibronectin were expressed as a ratio of the optical density units for either TGF-β1 or fibronectin to GAPDH.

Immunohistochemical labeling

GEC grown on glass coverslips were exposed to ox-LDL (50 μg/ml) for 24 hours. Immunoperoxidase staining was performed using the avidin-biotin complex method as previously described [44]. GEC on coverslips were rinsed three times with ice-cold phosphate-buffered saline (PBS), pH 7.4, and fixed with 100% methanol for 5 to 7 minutes at −20°C. The fixed coverslips were rinsed three times with PBS, and then incubated with 1.5% normal goat serum for 20 minutes at 25°C, followed by incubation with primary antibodies, either rabbit polyclonal anti-human TGF-β antibody (1:250; R&D Systems) or rabbit polyclonal anti-human fibronectin (1:500; Chemicon International Inc., Temecula, CA, USA) for 60 minutes at 25°C. After removing unbound primary antibody and rinsing with PBS, coverslips were incubated with biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA, USA) for 60 minutes at 25°C.

Coverslips were rinsed and then incubated with avidin-biotinylated horseradish peroxidase (Vectastain Elite ABC Kit; Vector) for 30 minutes at 25°C, followed by incubation (5 min at 25°C) with 0.1% diaminobenzidine tetrahydro-chloride (Sigma) in 0.1 mol/liter Tris buffer, pH 7.6, containing 0.02% H_2O_2. Finally, coverslips were washed in tap water and mounted with Permount® (Fisher Scientific, Pittsburg, PA, USA). Negative controls consisted of substituting the primary antibody with nonspecific rabbit IgG or PBS.

Analytical studies

Results were expressed as means ± SE for three separate experiments. Statistical analysis were performed using Student's *t*-test with $P < 0.05$ taken as significant.

Results

Effect of ox-LDL on TGF-β mRNA expression in GEC

The effect of increasing concentrations of ox-LDL on TGF-β gene expression in GEC during 12 hours incubation is shown in Figure 1. Untreated cells express TGF-β mRNA in low steady-state levels, whereas GEC treated with ox-LDL exhibited increased expression of TGF-β mRNA in a concentration-dependent manner between 25 and 100 μg/ml (113 +14%, 151 ± 10%, 180 ± 6%, and 195 ± 22% of control for ox-LDL 25, 50, 75, and 100 μg/ml, respectively). We then evaluated the time course during which ox-LDL could affect TGF-β gene expression. As shown in Figure 2, ox-LDL (50 μg/ml) increased steady-state TGF-β mRNA levels. Up-regulated expression appeared at six hours (210 ± 74% of control), and remained elevated at nine hours (163 ± 16% of control, $P < 0.05$) and 12 hours (217 ± 56% of control). At 24 hours, the TGF-β message was still elevated (285 ± 66% of control, $P < 0.05$).

In comparison with ox-LDL, incubation of GEC with native LDL led to the TGF-β message increase in a less pronouced dose-dependent fashion between 25 and 150 μg/ml (105 ± 2%, 120 ± 9%, 155 ± 18%, and 157 ± 44% of control for native LDL 25, 50, 100, and 150 μg/ml, respectively). Native LDL (100 μg/ml)-induced TGF- β mRNA increase appeared at three hours (126 ± 9% of control, $P < 0.05$) and peaked at 12 hours (145 ± 8% of control, $P < 0.05$). At 24 hours, the message level was 139 ± 27% of control. There was little or no difference in TGF-β message levels of GEC cultured between time 0 and 24 hours in the absence of native LDL or ox-LDL (data not shown). Since ox-LDL was more potent in stimulation of TGF-β mRNA expression, all the subsequent experiments was carried out with ox-LDL.

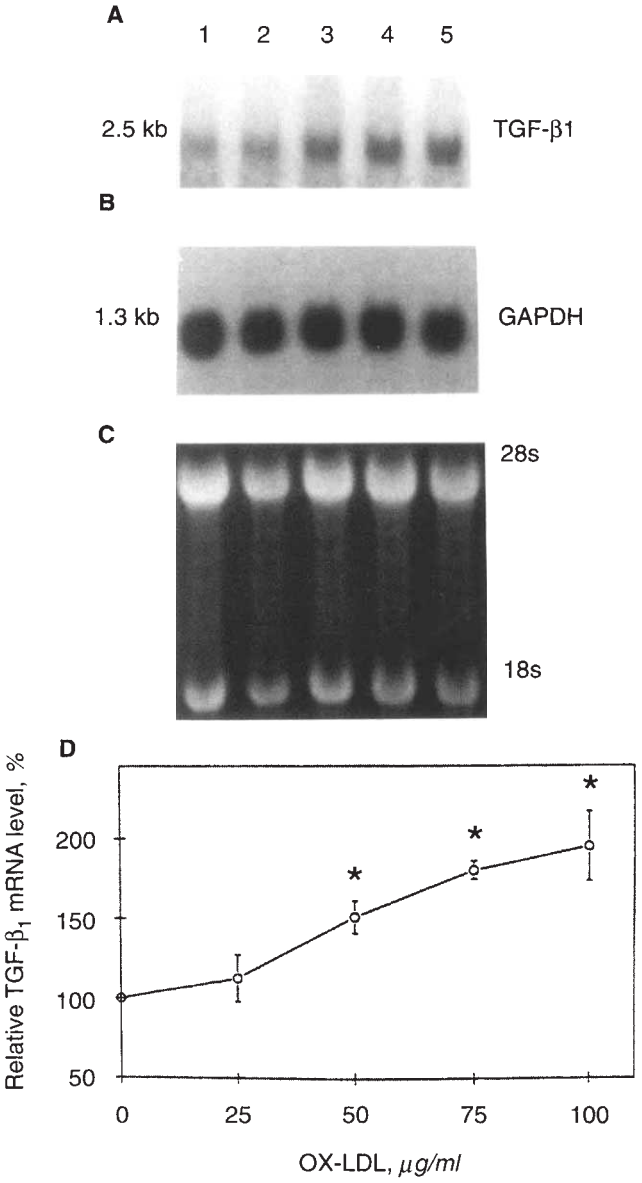

Fig. 1. *Dose response of TGF-β mRNA expression from ox-LDL-treated human glomerular epithelial cells (GEC).* Northern blotting analysis of total RNA from control GEC (lane 1) and GEC treated with different concentrations of ox-LDL for 12 hours: 25 μg/ml (lane 2), 50 μg/ml (lane 3), 75 μg/ml (lane 4), and 100 μg/ml (lane 5). Blots were probed for TGF-β1 (**A**) and then for GAPDH (**B**). **C** shows 28 s and 18 s rRNA. **D**. quantitative expression of TGF-β1 mRNA abundance after correcting for the GAPDH signal. The TGF-β mRNA levels of treated GEC are expressed as % increases above the mRNA levels of untreated controls. Values are presented as the mean ± SE of three separate experiments.

Effect of ox-LDL on fibronectin mRNA levels in GEC

In the next series of experiments, the effect of ox-LDL on fibronectin message expression was assessed. GEC fibronectin mRNA levels were determined in cells cultured in the presence of ox-LDL (50 μg/ml). As shown in Figure 3, GEC constitutively expressed fibronectin-specific mRNA. An increase in fibronectin message was seen at 6 to 24 hours treatment with ox-LDL. The maximum stimulatory effect was observed at 24 hours post-treatment (325 ± 33% of control, $P < 0.05$).

Ox-LDL-stimulated TGF-β and fibronectin protein expression by immunocytochemistry

Since ox-LDL stimulated TGF-β and fibronectin mRNA expression in GEC, we evaluated the effects of ox-LDL on TGF-β and fibronectin protein production in GEC treated with or without ox-LDL (50 μg/ml). As shown in Figure 4, faint staining for TGF-β and fibronectin proteins were seen in untreated cells (Fig. 4 A, A′). Exposure to ox-LDL increased the intensity of

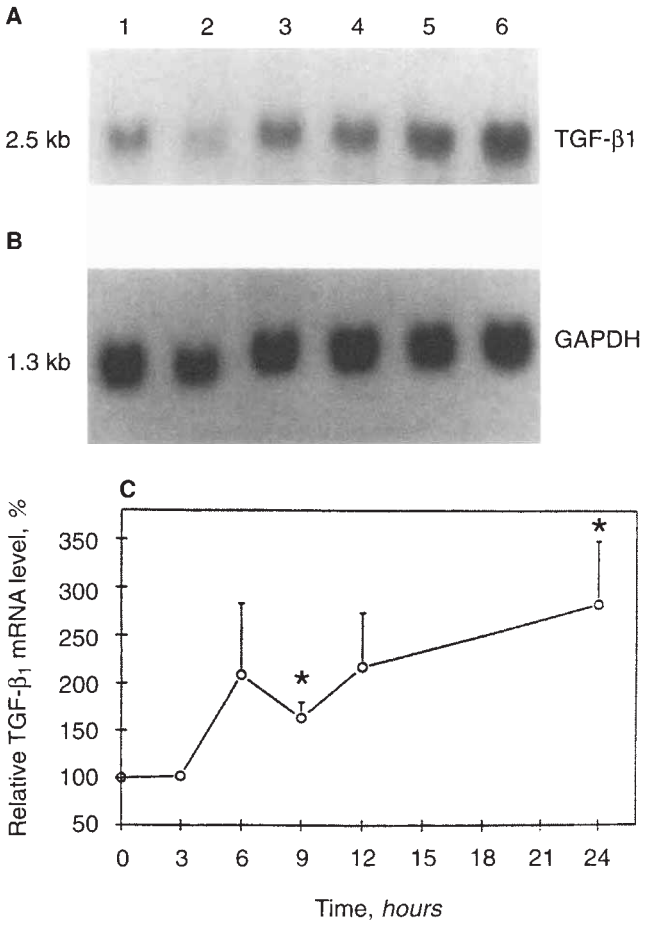

Fig. 2. *Time course of TGF-β mRNA expression from ox-LDL-treated GEC.* Northern blotting analysis of total RNA from the control cells (lane 1) and cells treated with ox-LDL (50 μg/ml) for three hours (lane 2), six hours (lane 3), nine hours (lane 4), 12 hours (lane 5), and 24 hours (lane 6). Blots were probed for TGF-β1 (**A**) and GAPDH (**B**). **C**. Quantitative expression of TGF-β1 mRNA abundance after correcting for the GAPDH signal. Data are expressed as described in Figure 1 legend. $^*P < 0.05$ versus control (representative of 3 separate experiments).

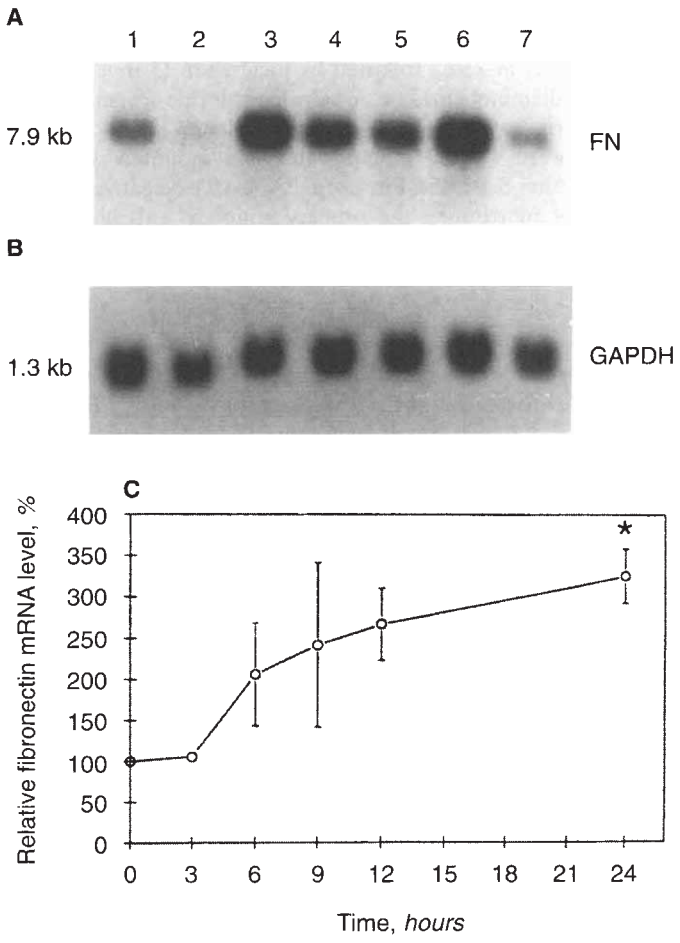

Fig. 3. *Expression of fibronectin (FN) mRNA from ox-LDL-treated GEC.* Northern blotting analysis of total RNA from GEC controls (lane 1 from 0 hr control and lane 7 from 24 hr control) and GEC treated with ox-LDL (50 μg/ml) for three hours (lane 2), six hours (lane 3), nine hours (lane 4), 12 hours (lane 5), and 24 hours (lane 6). Blots were probed for FN (**A**) and then for GAPDH (**B**). **C**. Quantitative expression of FN mRNA abundance after correcting for the GAPDH signal. Data are expressed as described in Figure 1 legend. $^*P < 0.05$ versus control (representative of 3 separate experiments).

staining for TGF-β (Fig. 4B) and fibronectin (Fig. 4B′). The enhanced fibronectin positivity appears to be cell associated. This may be related to the fact that the cells have been exposed to ox-LDL for relatively brief periods of time. Negative controls did not show increased staining (data not shown).

Effect of anti-TGF-β antibody on ox-LDL-stimulated expression of fibronectin mRNA

To investigate whether the newly-produced TGF-β was involved in the ox-LDL-stimulated fibronectin gene expression, GEC were incubated with ox-LDL (50 μg/ml) in the presence of rabbit anti-human TGF-β antibody (30 μg/ml) or non-specific rabbit IgG (30 μg/ml) for 6 and 24 hours. The fibronectin-specific mRNA was then analyzed by Northern hybridization. As shown in Figure 5, the addition of anti-TGF-β antibody for 24 hours significantly down-regulated fibronectin message expression in GEC treated with ox-LDL (139 ± 24% in ox-LDL+ anti-TGF-β vs. 217 ± 31% in ox-LDL, $P < 0.05$). In contrast, the addition of control rabbit IgG did not blunt ox-LDL-stimulated fibronectin mRNA expression (241 ± 35% in ox-LDL+ normal IgG vs. 217 ± 31% in ox-LDL). GEC co-treated with anti-TGF-β antibody and ox-LDL for six hours also exhibited a decrease in the mRNA level (96 ± 7% in ox-LDL + anti-TGF-β vs. 112 ± 6% in ox-LDL) (not shown).

Effects of inhibitors of gene transcription and protein synthesis on ox-LDL-stimulated TGF-β mRNA expression

GEC were co-treated with ox-LDL and the transcriptional inhibitor, actinomycin D (AMD,5 μg/ml), to determine whether transcriptional events are involved in the increase in TGF-β mRNA induced by ox-LDL. As seen in Figure 6, actinomycin D inhibited the ox-LDL-stimulated increase in TGF-β mRNA (ox-LDL+ AMD, 43 ± 7.0% of control, $N = 3$).

To assess the possible role of new protein synthesis in the action of ox-LDL, GEC were treated with ox-LDL and cycloheximide (CHX, 10 μg/mL), a protein synthesis inhibitor. When GEC were treated with cycloheximide for 12 hours, a significant increase in

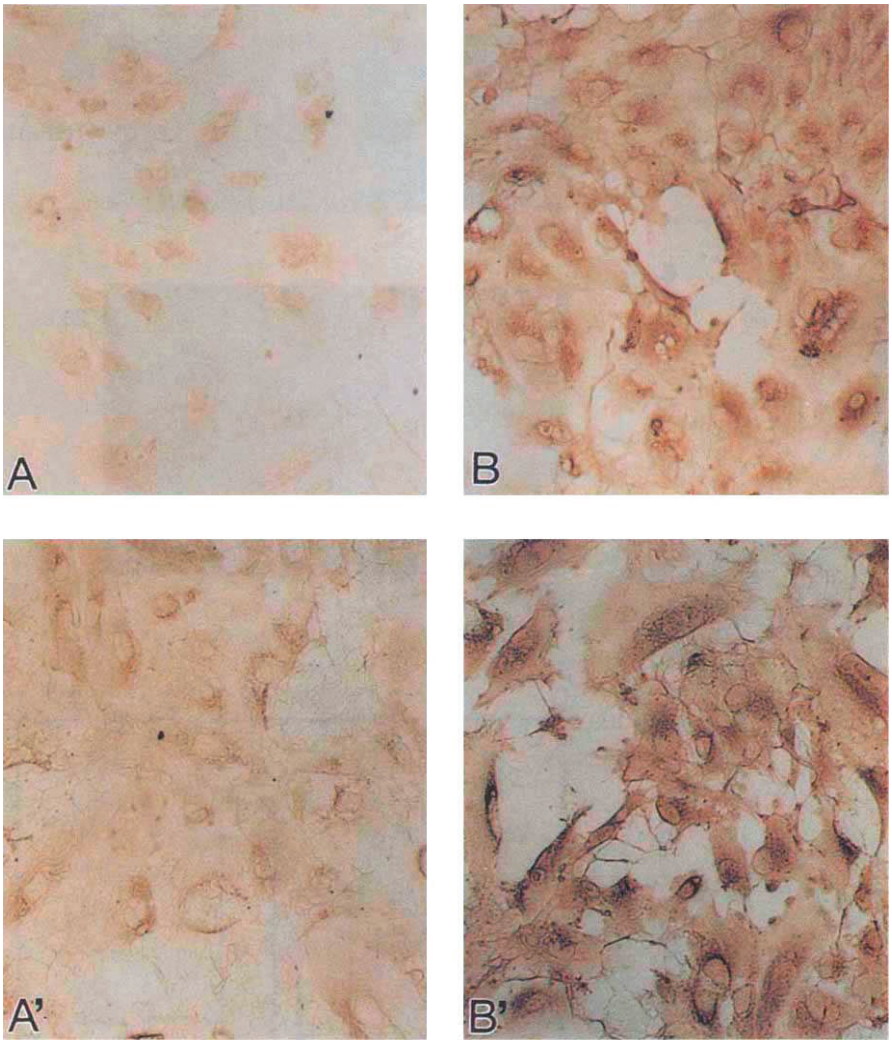

Fig. 4. *Immunoperoxidase staining of human GEC for TGF-β* (**A, B**) *and fibronectin* (**A′, B′**). A and A′ are the cells not exposed to ox-LDL. B and B′ are the cells exposed to ox-LDL (50 μg/ml) for 24 hours. TGF-β (B) and fibronectin (B′) were obviously increased in GEC exposed to ox-LDL. Comparable results were obtained in three sets of experiments.

fibronectin mRNA was observed (201 ± 26%, $P < 0.05$). On the other hand, treatment of GEC with cycloheximide in the presence of ox-LDL further increased TGF-β mRNA levels (ox-LDL + CHX, 303 ± 48% of control, $N = 3$).

Discussion

The pathogenic roles of atherogenic lipoproteins and glomerular cell alterations in structure and function have prompted investigations aimed at understanding the pathogenesis of lipoprotein-mediated glomerular injury. The present study shows that both ox-LDL and native LDL can stimulate TGF-β mRNA expression in cultured human GEC. Stimulation of GEC with ox-LDL caused an increase in TGF-β protein production as detected by immunocytochemistry. To our knowledge, the current investigation is the first demonstration that cultured glomerular epithelial cells express TGF-β, which appears to be a unique growth factor in its ability to stimulate glomerular extracellular matrix protein (ECM) production and deposition, ultimately leading to glomerulosclerosis [31].

Glomerular visceral epithelial cells are one of the major cell types within the glomerulus and play an important role in maintaining normal structure and function of the glomerular basement membrane (GBM). Significant changes in GEC morphology are frequently found in human and experimental glomerular diseases, particularly in the proteinuric state [45]. GEC proliferation *in vivo* has been observed in glomerular diseases such as focal and segmental glomerulosclerosis [46] and passive Heyman nephritis [47]. During nephrosis, GEC are directly exposed to increased concentrations of lipoproteins filtered through the Bowman's capsule.

Accumulation of lipids in GEC has been previously reported [10, 17, 18]. Gröne et al [19] recently demonstrated receptor-mediated uptake of LDL by cultured human GEC. Scavenger receptors, which can mediate the uptake of ox-LDL, are also present on the plasma membranes of human GEC as detected by immunocytochemical techniques [17], but their biological activity was not tested. Our current investigation demonstrates that both ox-LDL and native LDL stimulate TGF-β gene expression in human GEC. Ox-LDL is more potent than native LDL. The reason for this difference is not clear, but may be related to the state of oxidation of lipoproteins and/or quantitative differences in the activity of these two types of receptors. Recent investigations of Wheeler et al [27] revealed that incubation of LDL in culture medium alone led to oxidation of the particles from < 3 to 6.7 ±

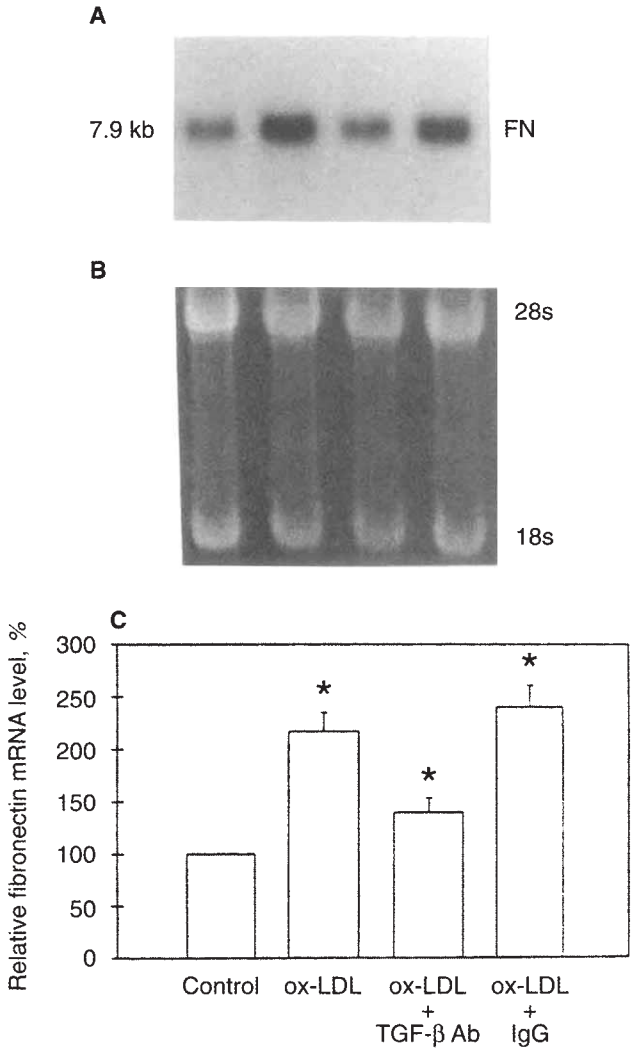

Fig. 5. *Effect of anti-TGF-β antibody on ox-LDL-stimulated expression of fibronectin mRNA.* Northern blotting analysis of total RNA from GEC control and GEC treated with ox-LDL in the absence or presence of anti-TGF-β antibody or non-specific rabbit IgG. Blots were probed for fibronectin (**A**). Intact 28 s and18 s rRNA bands are shown by ethidium bromide staining of agarose gel (**B**). **C**. Quantitative expression of FN mRNA levels as a ratio of the density reading of FN mRNA to that of the density of rRNA (28 s or 18 s) photograph from ethidium bromide stained formaldehyde gel. $^*P < 0.05$ versus control. $+P < 0.05$ versus ox-LDL-treated (representative of three separate experiments).

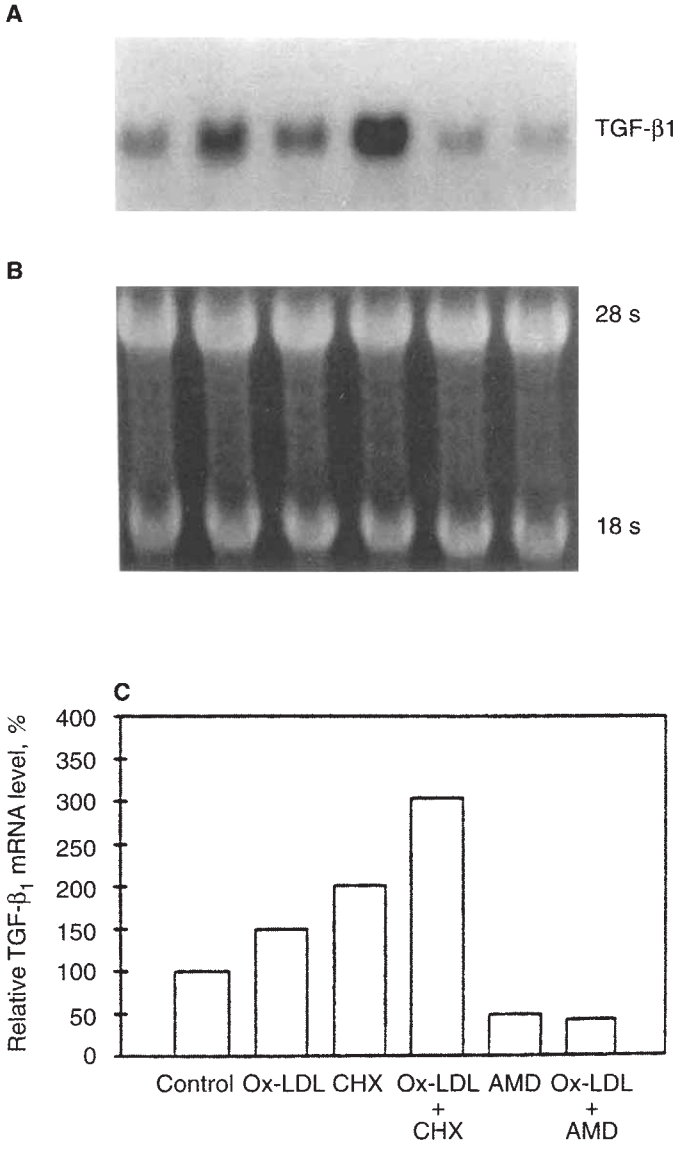

Fig. 6. *Effects of actinomycin D and cycloheximide on ox-LDL-stimulated TGF-β mRNA expression.* GEC were treated for 12 hours with actinomycin D (AMD) (5 μg/ml) or cycloheximide (CHX) (10 μg/ml), in the presence or absence of ox-LDL (50 μg/ml). Total RNA was extracted for Northern blotting analysis as described in the methods and Figure 5 legend (representative of 3 separate experiments).

1.0 nM MDA/mg LDL protein although cultured human GEC did not oxidize LDL up to 24 hours exposure. It is appealing to speculate that the less apparent response to native LDL may be mediated by a fraction that has been oxidized during the incubation. Studies of Coritsidis et al [25] have shown that rat mesangial cells preferentially bind and take up ox-LDL. Whether glomerular epithelial cells possess similar functioning receptors for ox-LDL remains to be clarified. However, our results are consistent with those reported for the effects of native LDL and ox-LDL on platelet-derived growth factor (PDGF) gene expression by smooth muscle cells [48], renin release by juxtaglomerular cells [49], and eicosanoid production by mesangial cells [25, 27].

Our studies also show that the ox-LDL-stimulated increase in the TGF-β transcript was diminished by actinomycin D, an inhibitor of gene transcription, suggesting that ox-LDL induces TGF-β mRNA synthesis at the transcriptional level. When GEC were co-treated with ox-LDL and cycloheximide, TGF-β mRNA levels were further increased when compared to GEC treated with ox-LDL alone. In addition, cycloheximide by itself increased TGF-β transcript levels. The mechanisms by which cycloheximide affects TGF-β mRNA are not known and were not addressed by the present work. However, the so-called "superinduction" of mRNA has been described previously in a variety of cell systems [50]. The mRNA superinduction is usually attributed to decreased mRNA turnover rate, or alternatively due to the lack of *de novo*

synthesis of transcriptional activator inhibitor, such as NF-κB inhibitor as demonstrated in the pulmonary epithelial cells [51].

TGF-β is a multifunctional growth factor that can either inhibit or stimulate cell proliferation and differention. This growth factor is generally considered to be the fibrogenic cytokine since it exerts a marked effects on ECM production [31]. The stimulatory effects of TGF-β on ECM accumulation have been shown *in vivo* by transfer of the TGF-β gene into the rat kidney [52].

An apparent relationship between increased renal TGF-β expression and ECM expansion has been demonstrated in various experimental and human glomerulopathies [31, 35, 53]. Resident glomerular cells possess high-affinity receptors for TGF-β and produce TGF-β [32, 54]. Previous studies in glomerular epithelial cells *in vitro* have shown that TGF-β is able to increase the production of several matrix proteins [34]. Exposure of GEC to TGF-β, in addition to up-regulating the synthesis of proteoglycans, increased the production of fibronectin, type IV collagen, and laminin. It has been suggested that GEC may be the cell type contributing the fibronectin and other nonproteoglycan components to the TGF-β-incuced ECM expansion in experimental glomerulonephritis [34]. The fact that GEC exposed to ox-LDL produces TGF-β, combined with the presence of high-affinity receptors for TGF-β on the cell surfaces of GEC strongly suggest that important interactions may occur in these cell types. In our GEC culture system, we subsequently evaluated the effects of ox-LDL on fibronectin expression. Ox-LDL increased the gene expression of fibronectin after six hours of treatment. The maximum stimulatory effect was found at 24 hours post-treatment. The increased protein synthesis of fibronectin was also demonstrated immunohistochemically. We further hypothesized that the newly-produced TGF-β in response to ox-LDL stimulates fibronectin expression. When GEC were exposed to ox-LDL in the presence of neutralizing antibody to TGF-β, we noticed an obvious reduction in fibronectin mRNA levels. These data support our hypotheses that ox-LDL stimulates the production of TGF-β, and that the newly-produced TGF-β is involved in increased fibronectin expression. Studies in glomerular mesangial cells revealed that LDL also stimulates fibronectin gene expression and protein synthesis [29]. LDL-stimulated fibronectin protein synthesis was completely blocked by the neutralizing antibody to TGF-β [28].

Although these *in vitro* experiments show a role for lipoprotein-induced growth factor production and fibronectin synthesis, the relevance of these findings to events that occur *in vivo* remains to be clarified. In renal diseases, such as focal glomerulosclerosis, apo B-containing lipoproteins were demonstrated in the visceral epithelial cells [17]. The *in vivo* deposition of lipoproteins may have effects similar to that of ox-LDL or LDL on human GEC *in vitro*.

In summary, the present study shows that ox-LDL stimulates the gene expression and protein production of TGF-β and fibronectin in cultured human GEC. The released TGF-β appears to be involved in the regulation of fibronectin expression in an autocrine fashion. Our data suggest that GEC is a potential source for the fibrogenic growth factor, TGF-β. Thus, the production of TGF-β by GEC may have important implications in the development of glomerulosclerosis.

Acknowledgments

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